PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07J 9/00 — A1

(11) International Publication Number: WO 97/42215

(43) International Publication Date: 13 November 1997 (13.11.97)

(21) International Application Number: PCT/US97/08296

(22) International Filing Date: 6 May 1997 (06.05.97)

(30) Priority Data:
60/016,966 6 May 1996 (06.05.96) US

(71) Applicant *(for all designated States except US)*: BIONUMERIK PHARMACEUTICALS, INC. [US/US]; Suite 1250, 8122 Datapoint Drive, San Antonio, TX 78229 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: HAUSHEER, Frederick, H. [US/US]; Suite 1250, 8122 Datapoint Drive, San Antonio, TX 78229 (US). HARIDAS, Kochat [IN/US]; Suite 1250, 8122 Datapoint Drive, San Antonio, TX 78229 (US).

(74) Agent: DODD, Thomas, J.; BioNumerik Pharmaceuticals, Inc., Suite 1250, 8122 Datapoint Drive, San Antonio, TX 78229 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: PROCESS FOR PREPARING 27-HYDROXY CHOLESTEROL AND RELATED DERIVATIVES

(57) Abstract

A process for preparing 27-hydroxy cholesterol and derivatives thereof. The process includes the conversion of the terminal acid or ester moiety to a reactive halomethyl moiety, which is eventually reduced to a hydroxy moiety after alkylation steps are performed.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

PROCESS FOR PREPARING 27-HYDROXY CHOLESTEROL AND RELATED DERIVATIVES

FIELD OF THE INVENTION

This invention relates to a novel and iterative process for producing 27-hydroxy cholesterol and its derivatives thereof. The process includes multiple steps in the conversion of cholenic acid or a derivative thereof to the desired title compound via a stereoselective alkylation methodology mediated by a oxazolidinone derived chiral auxillary using a novel triflate as key intermediate. The application of the present synthetic strategy is multi directional. Given the fact that the stereochemistry at C-25 determines the production of either 26- hydroxy cholesterol or 27- hydroxy cholesterol, one can carefully select the chiral auxillary either as its (S)- form or (R)- form which acts as a "stereoselective-switch" for the creation of the former S- isomer or the latter R-isomer using the same asymmetric alkylation methodology.

BACKGROUND OF THE INVENTION

27-hydroxy cholesterol is a well-known compound which has been clinically proven to raise levels of high density lipids (HDL) while lowering the levels of low density lipids (LDL) in humans. Commonly assigned United States Patent

Application Serial Number 08/436,034, filed May 5 ,1995, discloses methods of treating hypercholesterolemia and atherosclerosis by administering effective amounts of 27-hydroxy cholesterol to mammals having one of the above conditions.

Atherosclerosis and its associated complications, particularly coronary heart disease are the major health problems in developed countries worldwide. Certain risk factors, which include smoking, diabetes, hypertension, family history and low HDL, are associated with the development of atherosclerosis and coronary heart disease. Among these risk factors, plasma lipoproteins are important factors that affect the development of atherosclerosis. Elevated levels of low density lipoproteins (LDL) and reduced levels of high density lipoproteins (HDL) are associated with more severe atherosclerosis in humans and experimental animals, and with greater risk of coronary heart disease.

Both HDL and total cholesterol concentrations in the plasma of individuals vary considerably and are influenced by a number of factors, such as age, sex, diet, exercise, genetic deficiency. Among these factors, genetic and dietary factors have been suggested to play important roles in regulating HDL levels in plasma. However, very little is known about the mechanisms that regulate plasma HDL levels and how these mechanisms are affected by diets enriched in cholesterol and saturated or polyunsaturated fats.

The negative correlation between HDL cholesterol levels and atherosclerosis is believed to be due to the role of HDL in the reverse cholesterol transport process. According to this process, which was originally proposed by Glomset (Glomset, J.A., The plasma lecithin:cholesterol acyltransferase reaction. Journal of Lipid Research 9:155-167, 1968), HDL removes cholesterol from the peripheral tissues including the arterial wall and delivers it to the liver for excretion. A number of in vitro studies suggest that HDL and its subfractions enhance removal of cellular cholesterol. In this reaction, cholesterol derived by HDL removal from the tissues is esterified by lecithin:cholesterol acyltransferase (LCAT) and is transferred to triglyceride-containing lipoproteins in exchange for triglycerides. This reverse transfer of cholesterol from tissues is mediated in part by CETP in the plasma. The inventors submit that augmentation of reverse cholesterol transport by inhibiting, directly or indirectly, CETP mediated cholesterol ester transfer will result in augmenting HDL mediated reverse cholesterol transport from tissues. The inventors further submit that this approach is predicted to have substantial therapeutic utility for atherosclerosis, hypercholesterolemia and diseases related to endothelial dysfunction in humans.

HDL particles are characterized by three major subclasses (HDL_1, HDL_2, HDL_3) on the basis of their flotation rates. These subclasses of HDL are heterogeneous in

particle size and protein, cholesterol and triglyceride composition. The HDL particles are also heterogeneous in apolipoprotein content. Particles with apo A-I and A-II and particles with apo A-I but no apo A-II have been described . Cholesterol efflux from cultured adipose cells is mediated by LpAI particles but not by LpAI-AII particles. It has been suggested that HDL particles containing apo A-I mediate the transfer of cholesterol from cultured adipose cells whereas HDL particles with apo A-I and apo A-II do not. Thus different subclasses of HDL probably function differently and may differ in their antiatherogenic properties.

The role of CETP in the regulation of plasma HDL concentration was first recognized from studies of subjects with hyperalphalipoproteinemia. Koizumi et al. (Koizumi, J., Mabuchi, H., Yoshimura, A., Michishita, I., Takeda, M., Itoh, H., Sakai, Y., Nuda, K., and Takeda, R., Deficiency of serum cholesteryl ester transfer activity in patients with familial hyperalphalipoproteinemia. Atherosclerosis 58:175-186, 1985) reported two hyperalphalipoproteinemic subjects with a large HDL fraction that was clearly separated from LDL. The plasma from these subjects lacked CETP activity. Brown et al. (Brown, M.L., Inazu, A., Hesler, C.B., Agellon, L.B., Mann, C., Whitloc, M.E., Marcel, Y.L., Milne, R.W., Koizumu, J., Mabuchi, H., Takeda, R., and Tall, A., Molecular basis of lipid transfer protein deficiency in a family with increased high-density lipoproteins. Nature 342:448-451, 1989) later showed that

the familial deficiency of CETP activity was due to a gene splicing defect. Yokoyama et al. (Yokoyama, S., Kurasawa, T., Nishikawa, O., and Yamamoto, A., High density lipoprotein with poor reactivity in a homozygote of familial hyperalphalipoproteinemia. Artery 14:43-51, 1986) similarly reported that a homozygous subject with familial hyperalphalipoproteinemia had impaired plasma cholesteryl ester transfer between HDL and LDL. They also reported that the plasma fraction (d > 1.21 g/ml) from this subject had substantial transfer activity with normal HDL, but the HDL from this subject was a poor substrate for cholesteryl ester transfer. Subjects with a deficiency of CETP activity have been reported to also accumulate an LDL species not present in plasma of normal subjects.

HDL from subjects with hyperalphalipoproteinemia differed from HDLc in that it did not inhibit binding of LDL to LDL receptors in cultured human fibroblasts. Thus, the mechanism of accumulation of HDL in human CETP deficiency differs from that of HDLc . As in humans (described above), the accumulation of HDL_1 in high HDL_1 baboons was associated with slower transfer of cholesteryl esters from HDL to VLDL+LDL, due to an inhibitor. In addition to HDL_1, high HDL_1 baboons accumulate VLDL and LDL in their plasma in spite of a higher level of hepatic mRNA for LDL receptor compared to low HDL_1 baboons with similar levels of plasma LDL. Thus, the CETP activity also seems to affect HDL

concentration in the plasma of baboons in some sire families.

Increased plasma cholesterol and atherosclerosis is associated with abnormal endothelial function of coronary arteries (Harrison, D.G., Freiman, P.C., Armstrong, M.L., Marcus, M.L., Heistad, D.D., Alterations of vascular reactivity in atherosclerosis. Circulation Research 61:II-74-II-80, 1987). Abnormal endothelial function is commonly called endothelial dysfunction and this abnormality of the coronary endothelium precedes atherosclerosis and is believed to be a more sensitive marker for coronary risk (Harrison, D.G., Freiman, P.C., Armstrong, M.L., Marcus, M.L., Heistad, D.D., Alterations of vascular reactivity in atherosclerosis. Circulation Research 61:II-74-II-80, 1987; McLenachan, J.M., Williams, J.K., Fish, D., Ganz, P., Selwyn, A.P., Loss of flow-mediated endothelium-dependent dilation occurs early in the development of atherosclerosis. Circulation 84:1273-1278, 1991).

Coronary endothelial dysfunction may be detected by intraarterial infusion of acetylcholine (Ludmer, P.L., Selwyn, A.P., Shook, T.L., et al., Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. New England Journal of Medicine 315:1046-1051, 1986; Vita, J.A., Treasure, C.B., Nabel, E.G., et al., Coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. Circulation 81:491-497, 1990). An intraarterial infusion of

acetylcholine in normal coronary arteries with normal endothelium produces vessel dilation, whereas the infusion of acetylcholine into arteries with dysfunctional endothelium produces vasoconstriction (Ludmer, P.L., Selwyn, A.P., Shook, T.L., et al., Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. New England Journal of Medicine 315:1046-1051, 1986).

The vasodilation effects of intraarterial acetylcholine is mediated by the release of an endothelial cell derived vasorelaxant substance from the endothelium and has been recognized as nitric oxide (Guerra, R., Jr., Brotherton, A.F., Goodwin, P.J., Clark, A.R., Armstrong, M.L., Harrison, D.G., Mechanism of abnormal endothelium-dependent vascular relaxation in atherosclerosis: Implications for altered autocrine and paracrine functions of EDRF. Blood Vessels, 26:300-314, 1989; Bruckdorfer, K.R., Jacobs, M., Rice-Evans, C., Endothelium-derived relaxing factor (nitric oxide), lipoprotein oxidation and atherosclerosis. New England Journal of Medicine 18:1061-1063, 1990).

It has been reported that dysfunctional coronary endothelium produces lower amounts of nitric oxide as compared to the normal endothelium. It has also been reported that increases in plasma LDL adversely affects the production and release response of nitric oxide; these events result in dysfunctional coronary endothelial responses. Dysfunctional coronary endothelium promotes

platelet and leukocyte local aggregation in the coronary vessels and promotes monocyte or macrophage retention in coronary vessels; all of these events may lead to local damage to the coronary anatomor and increase the risk of developing coronary atherosclerosis (Levine, G.N., Keqny, J.F., Jr., Vita, J.A., Cholesterol reduction in cardiovascular disease. New England Journal of Medicine 332:512-521, 1995). The dysfunctional endothelium is often the cause of unstable angina and is related to restenosis of coronary vessels following percutaneous transluminal coronary angioplasty.

Several recent reports in human subjects have described that pharmacologic intervention with drugs that lower plasma levels of LDL cholesterol has resulted in restoration of normal endothelium dependent relaxation of coronary arteries (Treasure, C.B., Klein, J.L., Weintraub, W.S., et al., Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. New England Journal of Medicine 332:481-487, 1995; Harrison, D.G., Armstrong, M.L., Freiman, P.C., Heistad, D.D., Restoration of endothelium-dependent relaxation by dietary treatment of atherosclerosis. The Journal of Clinical Investigation 80:1808-1811, 1987; Osborne, J.A., Lento, P.H., Siegfried, M.R., Fusman, B., Lefer, A.M., Cardiovascular effects of hypercholesterolemia in rabbits. Reversal with lovastatin treatment. The Journal of Clinical Investigation 83:465-473, 1989; Levine, G.N., Keaney, J.F.,

Jr., Vita, J.A., Cholesterol reduction in cardiovascular disease. Clinical benefits and possible mechanisms. New England Journal of Medicine 332:512-521, 1995; Egashira, K., Takeshitam A., Beneficial effect of cholesterol-lowering therapy on endothelium-dependent coronary vasodilation in patients with hypercholesterolemia. Annuals of the NY Academy of Sciences 748:622-625, 1995; Egashira, K., Hirooka, Y., Kai, H., et al., Reduction in serum cholesterol with pravastatin improves endothelium-dependent coronary vasomotion in patients with hypercholesterolemia. Circulation 89:2519-2524, 1994; Leung, W., Wong, C., Beneficial effect of cholesterol-lowering therapy on coronary endothelium-dependent relaxation in hypercholesterolemic patients. Lancet 341:1496-1500, 1993; Gould, K.L., Martucci, J.P., Goldberg, D.I., et al., Short-term cholesterol lowering decreases size and severity of perfusion abnormalities by position emission tomography after dipyridamole in patients with coronary artery disease. A potential noninvasive marker of healing coronary endothelium. Circulation 89:1530-1538, 1994).

The exact mechanism of the restoration of normal endothelium function by plasma LDL reduction in humans is not known. However, it is likely that the decrease in LDL leads to reduced cholesterol deposition on the endothelial surface and possibly in some improvement in reverse cholesterol transport from peripheral tissues. The present invention is aimed at normalizing endothelial dysfunction by

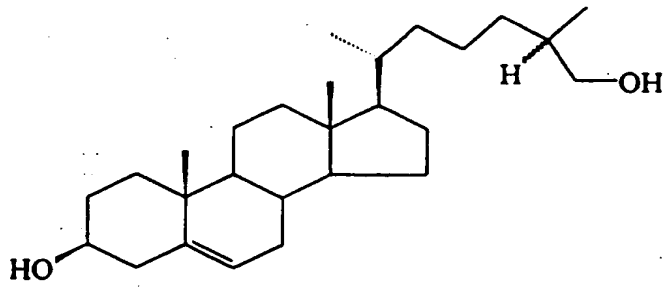
enhancement or augmentation of reverse cholesterol transport in endothelial cells using novel agents which directly increase HDL and augment reverse cholesterol transport or indirectly by increasing HDL plasma levels alone or in combination with LDL reduction by mechanisms which are poorly understood at the present time. The inventors submit that the increase in plasma HDL will also lead to an increase in reverse cholesterol transport, although this has not been demonstrated in human subjects. The inventors further submit that the increase in plasma HDL due to the administration of pharmacologically active levels of CETP inhibitors and/or HDL elevating drugs which are the subject of this invention will normalize coronary endothelial function and will improve patient outcomes, such as morbidity and mortality, from conditions such as unstable angina, and/or will retard, prevent or lessen the incidence of restenosis in patients who have undergone angioplasty procedures. The inventors also predict that the use of the new pharmacologic agents as described in the present invention will prevent, retard or substantially reduce the early damage to the coronary endothelium which arises from increased plasma LDL levels in human subjects. The inventors also wish to point out that the pharmacologic agents in the present invention can be combined with other known drugs, such as those known to predominantly lower plasma LDL in humans; such combination therapy is predicted to have significant clinical utility by increasing reverse

cholesterol transport (HDL mediated) and reducing cholesterol ester deposition (LDL mediated) which would lead to more rapid, or the preservation of, normalization of coronary endothelial function in human subjects at risk for the development of this problem.

SUMMARY OF THE INVENTION

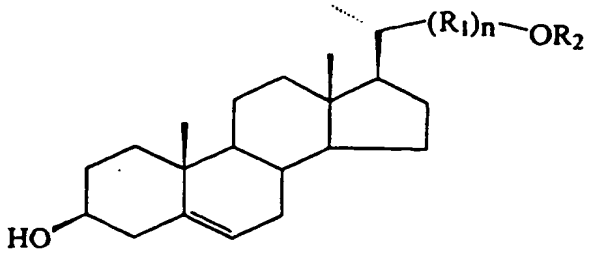
The invention discloses and claims a useful, novel and non-obvious process for producing 27-hydroxy cholesterol and derivatives thereof. The process involves a multiple step iterative process which significantly reduces the cost of the desired final compounds and generates excellent yields compared to the prior art methods of synthesis.

27-hydroxy cholesterol has the following formula:



Derivatives of 27-hydroxy cholesterol which may be produced by the process of this invention have the formula:

(I)



wherein:

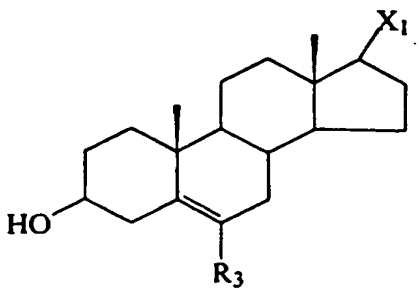
R_1 is a straight or branched hydrocarbon chain and n is 1 to 8; and

R_2 is hydrogen, C_1-C_6 alkyl or aryl.

The process of this invention comprises the steps of:

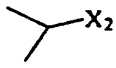
(i) providing a quantity of a starting material having the general formula;

(S)



wherein

R_3 is hydrogen, hydroxy or C_1-C_6 alkyl;

X_1 is oxo or  ; and

X_2 is hydroxy-(C_0-C_8 alkyl, C_2-C_{10} alkenyl, or C_2-C_{10} alkynyl), C_1-C_8 alkoxy, carboxy-(C_0-C_8 alkyl or C_2-C_{10} alkenyl), or alkoxycarbonyl (C_0-C_8 alkyl);

(ii) converting the starting material to cholenic acid or a protected cholenic acid or a derivative thereof;

(iii) reducing the terminal acid or ester moiety of the cholenic acid or protected cholenic acid to a terminal moiety which is reactive with a halomethyl or a halo derivative;

(iv) converting the reactive terminal moiety to a halomethyl or a halo moiety by reacting with a a halogenating reagent;

(v) converting the halomethyl or halo moiety to a chiral-bearing auxiliary adduct which is capable of generating an acid terminal moiety via reductive cleavage followed by a suitable metal hydride reduction

The inventive process includes novel alkylation steps which convert the starting materials to molecules having a terminal carboxylic ester or primary hydroxy moiety. These intermediates are then converted to a reactive triflate terminal moiety or a halomethyl functionality. After the site directed alkylation the alkylated adduct is then reduced to form the desired final compound, 27-hydroxy cholesterol or a derivative thereof either in one pot reaction or reduction followed by C5- oxo deprotection.

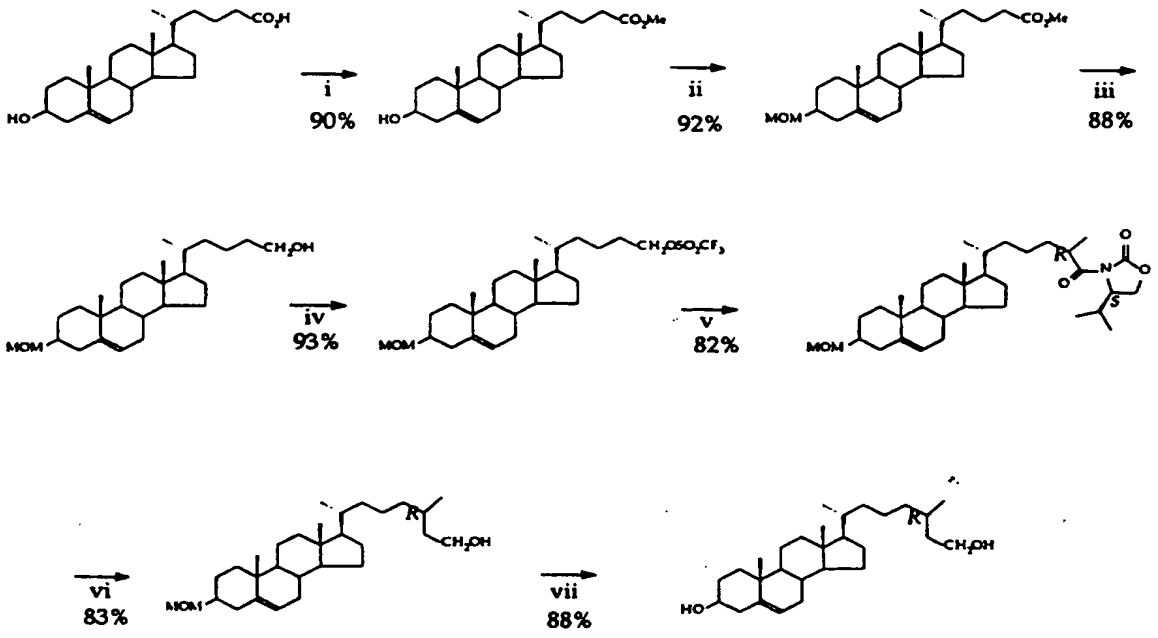
DETAILED DESCRIPTION OF THE INVENTION

The preferred embodiments herein described are not intended to be exhaustive, nor do they limit the invention in any way. They are chosen and described in order to explain the principles of the invention, so that others skilled in the art may apply its teachings to practice the invention.

The process of this invention is generally described in the following schemes.

SCHEME I

Stereoselective Synthesis of BNP9010



Reagents(i) MeOH/cat.H_2SO_4, r.t;(ii) MOMCl, CH_2Cl_2, Pyr, 0- 25°C; (iii) LAH, ehter, r.t; (iv) $(TfO)_2O$, 2, 6- lutidene, CH_2Cl_2, -78°C; (v) (s)-(+)- 4- isopropyl- 3- propionyl oxazolidinone NaHDMS, THF, -78 to -30; (vi) LAH, ether, 0°C; (vii) HCl, wet THF, 0°C to 25°C

As shown in Scheme I, the preferred starting material is 5- cholenic acid-3β-ol (**A**), which is a commercially available material. Cholenic acid is first protected by conversion to its 24- carboxylic ester (methyl ester **II** is shown) using the desired anhydrous alcohol in presence of catalytic amounts of a concentrated mineral acid. The ester (**II**) formed will correspond to the alcohol used. In the preferred embodiment, the acid (**A**) is converted to its

methyl ester (II) by using methanol in catalytic amounts of concentrated sulfuric acid. Other mineral acids or acidic catlysts and other alcohols may be used to create similar cholenic acid esters without departing from the spirit of this invention.

The 3-hydroxy group is then protected in a standard fashion. The preferred method discloses conversion of the 3-hydroxy group to a methoxymethoxy moiety by the use of methoxymethoxy chloride (MOM-Cl) in presence of a suitable organic base such as diisopropyl ethyl amine or pyridine to create the diprotected intermediate (III). Organic solvents are preferred in this step. Most preferred is dichloromethane at room temperature or slightly lower than room temperature (0° C-25°C).

The diprotected intermediate (III) is then reduced to a 23-hydroxymethyl intermediate (IV) by use of a commonly employed reducing agent. Preferred is lithium aluminum hydride, but other reducing agents can be used and are known to those skilled in the art.

23-hydroxymethyl intermediate (IV) is then converted through a two-step process into the 3-protected 27-hydroxy cholesterol intermediate (VII). First, intermediate (IV) is converted to a triflate or a reactive halide intermediate (V) by reacting (IV) with a triflylating reagent or a halogenating reagent. Such as a triflylating reagents include triflic anhydride or a suitable phenyl triflylamide. The preferred reagent is most preferably trifluoromethyl

sulfonic anhydride at reduced temperatures. Preferred halogenating reagents include phosphrous tribromide in presence of a suitable organic base or triphenylphosphine-iodine system in acetonitrile- ether in presence of a suitable organic base.

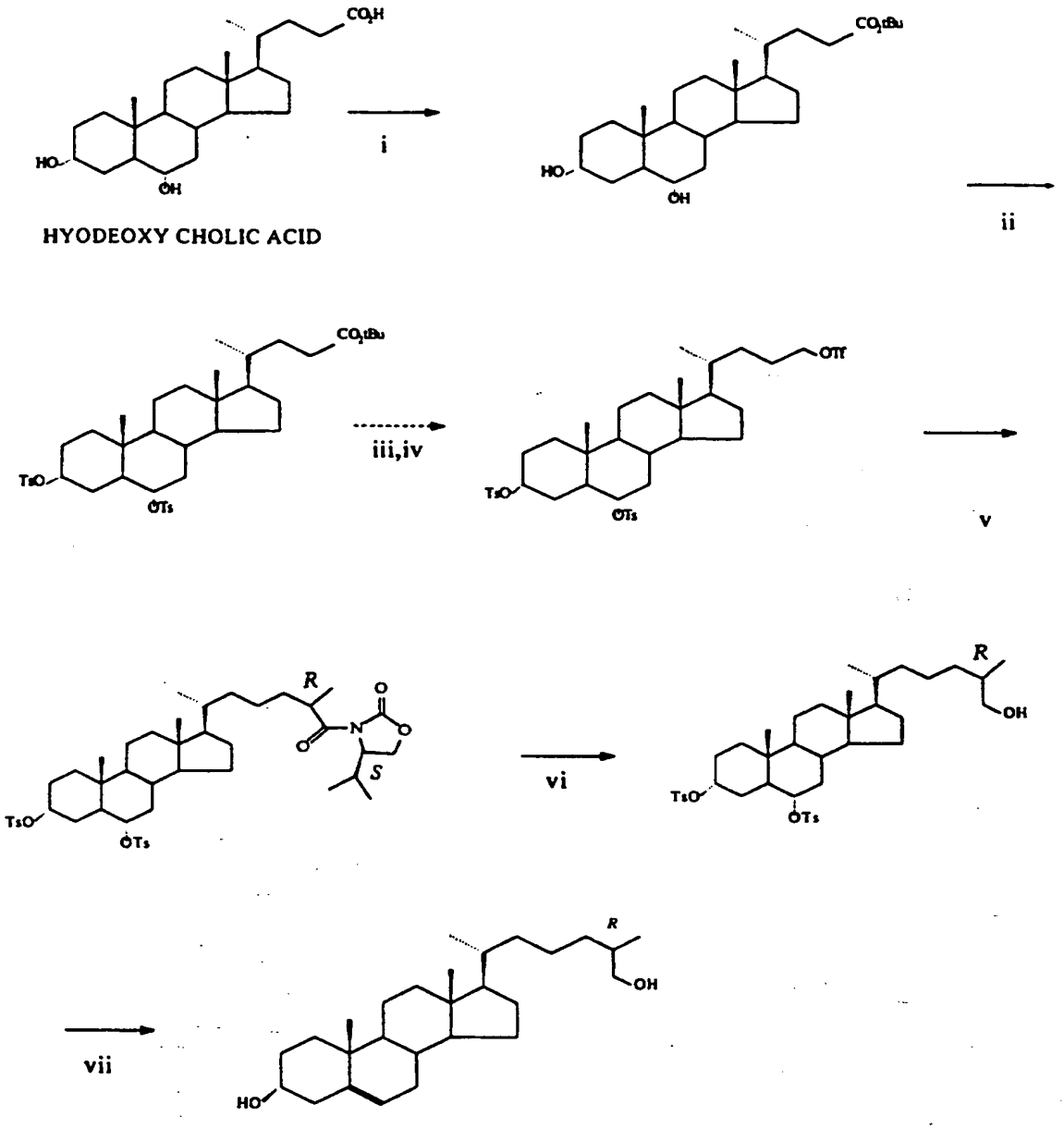
The triflate Intermediate (V) is then converted to a chiral auxiliary-bearing adduct (VI) which can be then converted to a steroidal carboxylic acid. A preferred reagent for this key step of alkylation is any reagent which will effect a stereoselective displacement of the triflate moiety to form a three carbon aliphatic chiral unit. In particular, an *in situ* generated Evan's enolate solution is most preferred for this step of the process. However, any known reagent which will effect chiral induction on the triflate terminal moiety may be used without departing from the spirit of the invention. Specific details of the best mode of carrying out the process of this invention are set forth in the Examples section of this specification, *supra*.

Next, the chiral adduct (VI) is reductively cleaved to the protected 27-hydroxy cholesterol intermediate (VII). As with step iii, any reducing agent may be used, with the preferred being lithium aluminum hydride or lithium borohydride. Finally, the intermediate (VII) is deprotected by standard methods, preferably with an acid to form 27-hydroxy cholesterol (I).

The process as shown in Scheme I is useful for preparing 27-hydroxy cholesterol or a derivative thereof. Specific examples of the process, which illustrate the preparation of individual derivatives, are included later in this specification.

It should be noted that the stereoselective process depicted above may be utilized to prepare either a specific stereoisomer of the Formula I compound, or may be used to produce a racemic mixture of diastereomers, as desired. Selection of reagents throughout steps iv and v will determine the structure of the final compound, as well as its relative stereochemistry.

SCHEME II



REAGENTS: (i) Isobutylene, conc. H_2SO_4, Et_2O, 25oC (ii) TsCl, Pyr., (iii) TFA, CH_2Cl_2, (iv) LAH, Et_2O (v) Tf_2O, Hunnig's base (vi) (s)-(+)- 4- isopropyl- 3- propionyl oxazolidinon NaHDMS, THF, -78 to -30 oC; (vi) LAH, ether, 0 oC (vii) NaOAc, DMF, 120C

Scheme II illustrates an alternative process for making compounds of Formula I. As shown, the starting material for

this process is hyodeoxy cholic acid (VIII). Hyodeoxy cholic acid (VIII) is available commercially from Sigma® Chemicals at a very economic cost.

First, hyodeoxy cholic acid (VIII) is protected by common methods. In the subsequent step , the terminal carboxylic acid moiety is protected as *tert*-butyl ester. Preferred reagents for the protection step are isobutylene and a catalytic amount of a concentrated mineral acid, but this step may be performed in other ways with different reagents as described in the literature. Protection of the carboxylic acid forms the ester intermediate (IX).

Next, the hydroxy moieties are protected suitably as their corresponding substituted phenyl ethers or tosylates or as such derivatives where those protecting groups selected must be non-reactive while processes are performed on the 17-position side chain, and in addition, must allow for later removal of the entire 6-position moiety. Preferred for purposes of this description are tosylate moieties, however other protecting groups, known in the art, could be used as well.

The ester portion of the compound is then hydrolyzed back into its acid form by reacting with a strong organic acid (trifluoroacetic acid is one of the many acids which could be employed here, and is the preferred acid). Immediately following hydrogenolysis, the compound is reduced to the alcohol form as described in step iii of Scheme I, and then reacted with a triflylating or

halogenating reagent to form the intermediate triflate or halo derivative **(XI)**.

Next, intermediate **(XI)** is subjected to the auxiliary-bearing substitution and subsequent reduction to the protected 27-hydroxy cholesterol intermediate **(XII)** as described above in Scheme I.

Intermediate **(XII)** is then deprotected and the 6-oxytosylate moiety removed to produce the desired Formula I compound. Preferred reagents for the final deprotection and generation of unsaturation step are salts of organic acids, most preferred being sodium acetate in dimethylformamide (DMF) at elevated temperatures.

The following specific examples illustrate one preferred process which is currently used to produce Formula I compounds of this invention. These examples should in no way be construed as limiting the invention to a specific reagent(s), or conditions. The invention is defined by the claims which follow this description.

Example 1

24-Methyl-3β-hydroxy- 5-cholenate

Cholenic acid (500 mg, 1.33 moles) was dissolved in anhydrous methanol (150 ml) under an inert atmosphere. Concentrated sulfuric acid (0.1 ml; catalytic amount) was

added and stirred at room temperature for 24 hours by which time, the starting material had disappeared. Once the reaction was completed, the solvent was evaporated over a rotary evaporator to a slurry. The white residue thus obtained was then dissolved in chloroform (100 ml) and washed with water (25 ml) and dried over granular anhydrous sodium sulfate. The organic portion was then concentrated to obtain the title compound in quantitative yield.

^{1}H NMR: 0.66 δ(3H, s); 0.92 δ(3H, δ, J= 5 Hz); 1.02 δ (3H, s); 1.06 - 1.1.55 δ (m); 1.79 - 1.86 δ (m); 1.95- 2.2 δ (m); 2.26- 2.34 δ (m); 3.5 δ (1H, m); 3.65 δ (3H, s); 5.36 δ (1H, d, J= 5.5 Hz)

Example 2

24-Methyl-3β-O-methoxymethyl- 5- cholenate

The methyl ester of cholenic acid from Example 1 (8.6 gm, 22.1 mmoles) was taken up in anhydrous methylene chloride (250 ml) at room temperature. Anhydrous pyridine (4 ml) was added to the solution. The resultant solution was then cooled to 0° C using an ice bath under an inert atmosphere of argon and methoxymethyl chloride (4 ml) added in a dropwise manner. The reaction mixture was then stirred for 4 hours at low temperature followed by monitoring the

disappearance of the starting material. Once the reaction was completed, the reaction mixture was diluted with an excess of methylene chloride (200 ml) and washed with water (3 X 50 ml). The organic portion was then dried over anhydrous sodium sulfate and the solvent evaporated to obtain the crude product as a white mass. The crude product was then flashed over a column of silica gel using 2% methanol in chloroform to furnish 9.1 grams of the title compound.

^{1}H NMR: 0.66 δ (3H, s); 0.92 δ (3H, d, J= 5 Hz); 1.02 δ (3H, s); 1.06 - 1.1.55 δ (m); 1.79 - 1.86 δ (m); 1.95- 2.2 δ (m); 2.26- 2.34 δ (m); 3.35 δ (3H, s); 3.5 δ (1H, m); 3.65 δ (3H, s); 4.781 δ (2H, s); 5.36 δ (1H, d, J= 5.5 Hz)

^{13} C NMR: δ 11.86, 18.33, 19.39, 21.06, 24.28, 28.15, 31.1, 31.9, 35.42, 36.77, 37.28, 39.54, 39.55, 39.77, 42.43, 50.17, 51.56, 55.25, 55.84, 56.84, 76.57, 94.83, 121.85, 141.9, 175.1

Example 3

23-Hydroxymethyl-3β-O-methoxymethyl- 5- cholene

The ongoing methyl ester (8.89 gm, 21 mmoles) was dissolved in anhydrous diethyl ether (200 ml) and lithium

aluminum hydride (0.78 gm, 21 mmoles) added under a blanket of argon at room temperature. The resultant suspension was then stirred for 6 hours under an argon atmosphere. The reaction mixture was then quenched using an ice cold saturated solution of ammonium chloride (100 ml), and allowed to stand for 30 minutes, then filtered through a celite bed. The inorganic precipitate and the celite bed were then washed using excess diethyl ether (4 X 100 ml). The combined organic portion was then dried over anhydrous sodium sulfate, filtered and concentrated to obtain the title compound in substantial purity. The product was then recrystallized from 2% ethyl acetate in hexane to deliver 8.01 grams of the hydroxymethyl intermediate in crystalline form.

^{1}H NMR: 0.66 δ (3H, s); 0.92 δ (3H, d, J= 5 Hz); 1.02 δ (3H, s); 1.06 - 1.1.55 δ (m); 1.79 - 1.86 δ (m); 1.95- 2.2 δ (m); 2.26- 2.34 δ (m); 3.35 δ (3H, s); 3.5 δ (1H, m); 3.6 δ (2H, t, J= 6.1 Hz); 4.781 δ (2H, s); 5.36 δ (1H, d, J= 5.5 Hz)

Example 4

<u>24-O-trifluoromethyl sulfonyloxymethyl-3β-O-methoxymethyl- 5- cholene</u>

The hydroxymethyl intermediate (200 mg, 0.49 mmoles) obtained from the above reaction was dissolved in anhydrous methylene chloride (15 ml) and cooled down to -78° C using a dry-ice bath. To the above cooled solution was then added 2, 6-lutidine (0.09 ml) using a syringe under an atmosphere of argon followed by trifluoromethyl sulfonyl anhydride (0.1 ml, 0.6 mmoles). The reaction mixture was then stirred for 2 hours at -78° C under a blanket of argon followed by monitoring the disappearance of the starting material. Once the reaction was over, the reaction mixture was diluted using water (25 ml) and methylene chloride (200 ml). The reaction mixture was concentrated at room temperature to dryness and then added 80% hexane in diethyl ether to preferentially precipitate the triflyloxy- lutidene salt. The organic fraction containing the product is then concentrated and dried over anhydrous sodium sulfate to furnish the desired triflate as an off white mass. The triflate was then dried over high vacuum for 4 hours at room temperature and utilized for the subsequent step immediately. The yield of the reaction was found to be quantitative.

^{1}H NMR: 0.66 δ (3H, s); 0.92 δ (3H, d, J= 5 Hz); 1.02 δ (3H, s); 1.06 - 1.1 δ (m); 1.79 - 1.86 δ (m); 1.95- 2.2 δ (m); 2.26- 2.34 δ (m); 3.35 δ (3H, s); 3.5 δ (1H, m); 4.51 δ (2H, t, J= 6.6 Hz); 4.781 δ (2H, s); 5.36 δ (1H, d, J= 5.5 Hz)

^{13}C NMR ($CDCl_3$): δ 11.74, 18.38, 19.26, 20.94, 24.13, 25.94, 28.06, 28.84, 31.1, 31.81, 35.14, 37.19, 39.5, 50.1, 55.73, 56.69, 78.19, 94.75, 121.74, 140.91

Example 5

27-N-[(4-isopropyl)-oxazolidinyl]-3β-O-methoxymethyl- 5- cholestenone

To a -78° C solution of lithium hexamethyldisilylamide (LHMDS)-(5 ml, 1.2 mmole equivalent, 1 M solution in tetrahydrofuran) was added (dropwise using a cannula) a solution of (S)-(+)-4-isopropyl-3-propionyl-2-oxazolidinone (4.5 mmole, 833 mg) precooled to around 0° C in 5 ml anhydrous tetrahydrofuran. The reaction medium was stirred for one hour at -78° C. To the above solution was then added dropwise the ongoing cholenic triflate (2.36 gm, 4.3 mmole) in 10 ml anhydrous tetrahydrofuran. The reaction was then stirred for approximately 10 hours at the above temperature and then quenched with saturated ammonium chloride solution (10 ml). The aqueous fraction was then extracted with chloroform (50 ml X 5) and the combined organic portion was washed once with saturated sodium chloride (50 ml) and dried over anhydrous sodium sulfate. After filtration, the solvent was removed over a rotary evaporator and the crude product was flashed over a bed of

silica gel using 10% methanol/chloroform to obtain fairly pure oxazolidinone adduct. The product was then taken to the subsequent step without any further purification.

^{1}H NMR: 0.66 δ (3H, s); 0.92 δ (3H, d, J= 5 Hz); 1.05 - 1.08 δ (6 H, s); (1.02 δ (3H, s); 1.06 - 1.1 δ (m); 1.79 - 1.86 δ (m); 1.95- 2.2 δ (m); 2.37δ (1H, m); 2.26- 2.34 δ (m); 3.21 δ (2H, m); 3.35 δ (3H, s); 3.5 δ (1H, m); 4.23 δ(1 H, m); 4.781 δ (2H, s); 5.36 δ (1H, d, J= 5.5 Hz)

Example 6

25(R),27-Hydroxy-3β-O-methoxymethyl- cholesterol

The adduct (0.8 gram, 1.4 mmole) obtained from Example 5 was taken up in anhydrous ether (25 ml) and lithium aluminum hydride (53 mg, 1.4 mmole) was added at 0° C and stirred for one hour. The reaction mixture was then quenched with a saturated solution of ammonium chloride (100 ml), allowed to stand for 30 minutes and filtered through a celite bed. The inorganic precipitate and the celite bed was then washed using excess chloroform (4 X 100 ml). The combined organic portion was then dried over anhydrous sodium sulfate, filtered and concentrated to obtain the title compound in substantial purity. The product was then recrystallized from 2% ethyl acetate in hexane to deliver

the hydroxymethyl intermediate in crystalline form. The yield of this reaction was found to be 68%.

^{1}H NMR: 0.66 δ (3H, s); 0.92 δ (3H, d, J= 5 Hz); 1.02 δ (3H, s); 1.06 - 1.1.55 δ (m); 1.79 - 1.86 δ (m); 1.95- 2.2 δ (m); 2.26- 2.34 δ (m); 3.35 δ (3H, s); 3.6 δ (2H, dd, J= 6.4 Hz); 4.26 δ (1H, m); 4.781 δ (2H, s); 5.36 δ (1H, d, J= 5.5 Hz)

^{13}C NMR ($CDCl_3$): δ 11.75, 16.39, 16.62, 18.57, 19.26, 20.95, 23.35, 24.18, 28.16, 28.83, 31.8, 33.45, 33.58, 35.64, 35.72, 36.08, 36.18, 36.66, 37.16, 39.48, 39.7, 42.26, 50.1, 55.14, 56.07, 56.71, 68.35, 68.53, 94.71, 121.82, 140.85

Example 7

25(R),27-Hydroxy-3β-O-methoxymethyl-cholesterol

The adduct (1.6 gram, 2.8 mmole) obtained from Example 6 was taken up in anhydrous ethanol (25 ml) and lithium borohydride (3 mmole equivalent) was added at 0° C and stirred for 5 hours at room temperature. The reaction mixture was then quenched with a saturated solution of ammonium chloride (100 ml), allowed to stand for 30 minutes and filtered through a celite bed. The inorganic precipitate and the celite bed was then washed using excess chloroform (4 X 100 ml). The combined organic portion was

then dried over anhydrous sodium sulfate, filtered and concentrated to obtain the title compound in substantial purity. The product was then recrystallized from 2% ethyl acetate in hexane to deliver the hydroxymethyl intermediate in crystalline form. The yield of this reaction was found to be 68%.

^{1}H NMR: 0.66 δ (3H, s); 0.92 δ (3H, d, J= 5 Hz); 1.02 δ (3H, s); 1.06 - 1.1.55 δ (m); 1.79 - 1.86 δ (m); 1.95- 2.2 δ (m); 2.26- 2.34 δ (m); 3.35 δ (3H, s); 3.6 δ (2H, dd, J= 6.4 Hz); 4.26 δ (1H, m); 4.781 δ (2H, s); 5.36 δ (1H, d, J= 5.5 Hz)
^{13}C NMR ($CDCl_3$): δ 11.75, 16.39, 16.62, 18.57, 19.26, 20.95, 23.35, 24.18, 28.16, 28.83, 31.8, 33.45, 33.58, 35.64, 35.72, 36.08, 36.18, 36.66, 37.16, 39.48, 39.7, 42.26, 50.1, 55.14, 56.07, 56.71, 68.35, 68.53, 94.71, 121.82, 140.85

Example 8

25(R),27-Hydroxycholesterol

The 5β-Methoxymethoxy intermediate (1 gram) from the above reaction was stirred with methanol (20 ml) and 1 ml of 1 N hydrochloric acid at room temperature for 1 hour. The reaction mixture was saturated with sodium chloride and extracted with chloroform (20 ml X 5). The combined organic portion was then dried over anhydrous sodium sulfate, and

the solvent was filtered and evaporated to deliver the title compound in the crude form. The crude product was then flashed over a column of silica gel using 10% methanol in chloroform to obtain the pure product in 80% yield.

^{1}H NMR: 0.66 δ (3H, s); 0.92 δ (3H, d, J= 5 Hz); 1.02 δ (3H, s); 1.06 - 1.1.55 δ (m); 1.79 - 1.86 δ (m); 1.95- 2.2 δ (m); 2.26- 2.34 δ (m); 3.6 δ (3 H, m); 5.36 δ (1H, d, J= 5.5 Hz)
^{13}C NMR ($CDCl_3$): δ 11.75, 16.39, 18.62, 19.29, 20.96, 23.33, 24.18, 28.15, 31.55, 31.79, 33.43, 33.56, 35.7, 36.05, 36.16, 36.4, 37.15, 39.68, 42.22, 50.03, 56.03, 56.69, 68.34, 68.52, 71.77, 121.78, 140.85

Example 9

23-Bromomethyl-5β-O-methoxymethyl-cholenate

The ongoing hydroxymethyl intermediate from Example 3, above, (500 mg, 1.24 mmole) was dissolved in anhydrous methylene chloride (10 ml) and cooled down to 0° C and to it was added anhydrous pyridine (0.15 ml, 1.3 mmole) using a syringe followed by phosphorus tribromide in methylene chloride (1 M soln., 0.42 ml, 0.6 mmoles) and stirred for 2 hours. After 2 hours, the reaction mixture was diluted with water (20 ml) and the organic product was extracted using chloroform (50 ml X 3). It was then dried and filtered.

Upon concentration, the product was obtained as white powder in substantial purity. Therefore, further purification was avoided. The yield was found to be quantitative.

^{1}H NMR: 0.66 δ (3H, s); 0.92 δ (3H, d, J= 5 Hz); 1.02 δ (3H, s); 1.06 - 1.1.55 δ (m); 1.79 - 1.86 δ (m); 1.95- 2.2 δ (m); 2.26- 2.34 δ (m); 3.35 δ (3H, s); 3.5 δ (1H, m); 3.69 δ (2H, t, J= 6.3 Hz); 4.781 δ (2H, s); 5.36 δ (1H, d, J= 5.5 Hz)

Example 10

25(RS),27-carboxymethyl-3β-O-methoxymethyl-cholesterol

A solution of methyl propionate (0.053 ml, 0.55 mmole) was dissolved in anhydrous tetrahydrofuran (3 ml) and cooled to -78° C. Freshly prepared lithium diisopropyl amide solution (prepared from diisopropyl amine (0.092 ml, 0.694 mmole) and n-butyl lithium (2.5 M solution, 0.2 ml, 0.55 mmole)) was added at 0° C. The pale yellow solution was stirred for 30 minutes at 0° C. The reaction medium was then cooled to -78° C and the above bromomethyl intermediate (240 mg, 0.495 mmole) was added in 5 ml tetrahydrofuran freshly distilled over sodium-benzophenone ketyl. The reaction mixture was stirred for 2 hours and quenched with saturated solution of ammonium chloride (3ml). The organic portion was then extracted out using chloroform (50 ml X 3). The combined organic portion was then dried over anhydrous

sodium sulfate and the solvent evaporated to obtain the crude product. The product, after flashing through a column of silica gel using 10% methanol in chloroform, provided the pure desired product in 68% yield.

^{1}H NMR: 0.66 δ (3H, s); 0.92 δ (3H, d, J= 5 Hz); 1.02 δ (3H, s); 1.06 - 1.1.55 δ (m); 1.79 - 1.86 δ (m); 1.95- 2.2 δ (m); 2.26- 2.34 δ (m); 3.35 δ (3H, s); 3.6 δ (2H, dd, J= 6.1 Hz); 3. 68 and 3.69 δ (3H, s); 4.26 δ (1H, m); 4.781 δ (2H, s); 5.36 δ (1H, d, J= 5.5 Hz)

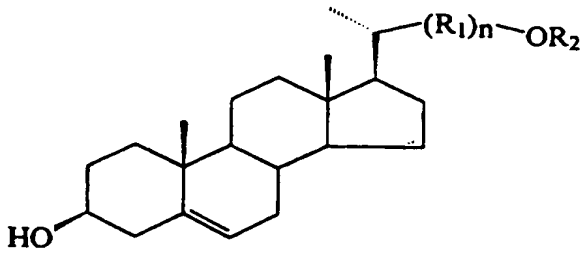
^{13}C NMR ($CDCl_3$): δ 11.75, 16.39, 18.62, 19.29, 20.96, 23.33, 24.18, 28.15, 31.55, 31.79, 33.43, 33.56, 35.7, 36.05, 36.16, 36.4, 37.15, 39.68, 42.22, 50.03, 56.03, 56.69, 68.34, 68.52, 71.77, 121.78, 140.85

The above examples are submitted as indicative of the process but in no way limit the invention to the precise materials or reaction conditions specified. The usefulness of the final desired products has been well-documented in the literature.

WHAT IS CLAIMED IS:

1. A process for making compounds having the following general formula:

(I)



wherein:

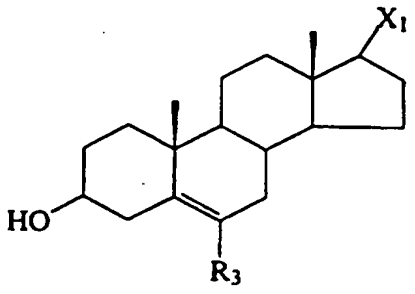
R_1 is a straight or branched hydrocarbon chain and n is 1 to 8; and

R_2 is hydrogen, C_1-C_6 alkyl or aryl;

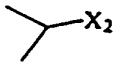
comprising the steps of:

(i) providing a quantity of a starting material having the general formula;

(S)



wherein R_3 is hydrogen, hydroxy or C_1-C_6 alkyl;

X_1 is oxo or  ; and

X_2 is hydroxy-(C_0-C_8 alkyl, C_2-C_{10} alkenyl, or C_2-C_{10} alkynyl), C_1-C_8 alkoxy, carboxy-(C_0-C_8 alkyl or C_2-C_{10} alkenyl), or alkoxycarbonyl-(C_0-C_8 alkyl);

(ii) converting the starting material to cholenic acid or a protected cholenic acid or a derivative thereof;

(iii) reducing the terminal acid or ester moiety of the cholenic acid or protected cholenic acid to a terminal moiety which is reactive with a halomethyl or a halo derivative;

(iv) converting the reactive terminal moiety to a halomethyl or a halo moiety by reacting with a triflylating reagent or a halogenating reagent;

(v) converting the halomethyl or halo moiety to a chiral-bearing auxiliary moiety which is capable of conversion to an acid terminal moiety; and

(vi) reductively cleaving the chiral-bearing auxiliary moiety to form the formula (I) compound.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/08296

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07J 9/00
US CL : 552/540, 542, 544

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 552/540, 542, 544

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,183,852 A (KAISER) 15 January 1980, see entire document	1
A	US 4,225,524 A (OCHI et al.) 30 September 1980, see entire document.	1
A	US 4,026,882 A (BAGGIOLINI et al.) 31 May 1977, see entire document	1
A	KOREEDA et al. Chirality Transmission via 6-Endo Free-Radical-Mediated Cyclization Process. Regio- and Stereocontrolled Synthesis of the 22 -Hydoxylated Steroid Side Chains. J. Am. Chem. Soc. November 1986, Vol. 108, No 25, pages 8098-8100.	1

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 29 JULY 1997	Date of mailing of the international search report 29 AUG 1997.
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JEAN F. VOLLANO Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

BNSDOCID: <WO___9742215A1_I_>

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/08296

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EGUCHI et al. Synthesis of 26,27-Dialkyl Analogues of 1α ,25-Dihydroxyvitamin D_3. Chem. Pharm. Bull. January 1988, Vol. 36, No. 7, pages 2303-2311. see entire document	1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/08296

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CAS ONLINE, CAS REACT

search terms, structure search in reg file and reaction structure search in CASREACT, hydroxy cholesterol, cholenic acid , chiral, oxazolidinone